Multifunctional MOF-based Electrochemiluminescent Nanocubes for an Ultrasensitive Biosensing Strategy of *B. Pseudomallei* Determination Coupled With 3D Magnetic Walking Nanomachine

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Abstract

Burkholderia pseudomallei (*B. pseudomallei*) can cause melioidosis that is usually fatal. A reliable and rapid detection method is greatly needed for disease surveillance and diagnosis. Herein, an ultrasensitive electrochemiluminescence (ECL) biosensor was constructed for accurate determination of *B. pseudomallei* coupled with multifunctional Au@Co-MOF@ABEI nanocubes and 3D magnetic walking nanomachine. The synthesized nanocubes could not only immobilize enormous ABEI but exhibit superior peroxidase-like activity to decompose H_2O_2 to produce plentiful reactive oxygen species (ROSs) that could further react with ABEI, so that the enhanced ECL signals were achieved. Meanwhile, the target-activated walking nanomachine was efficiently driven by Exonuclease III (Exo III) for further improving the sensitivity of the biosensor. As a result, the fabricated ECL biosensor could detect pathogenic gene down to 60.3 aM with a linear range from 100.0 aM to 100.0 pM. Moreover, the biosensing platform successfully achieved the determination of *B. pseudomallei* down to 9.0 CFU mL⁻¹ in serum samples. This work exhibited an ultrasensitive and specific performance for *B. pseudomallei* detection, which would become a versatile tool in the early diagnosis and treatment of melioidosis.

Introduction

B. pseudomallei, the pathogen of melioidosis, has been one of the most widespread bacteria responsible for extremely poisonous and tropical diseases [1–3]. It is estimated that *B. pseudomallei* is endemic in approximately 45 countries including multiple subtropical and tropical regions [4, 5]. Considering its higher fatality, early and sensitive detection of *B. pseudomallei* is urgently needed for disease surveillance and diagnosis melioidosis. At present, several traditional methods have been utilized for the determination of *B. pseudomallei*, such as conventional culture methods [6], polymerase chain reaction (PCR) [7], and enzyme-linked immunosorbent assay [8]. Although these approaches are powerful and reliable, most of them are expensive, labor-intensive, and time-consuming [9, 10]. Therefore, a detection method which can simple, cost-effective, and sensitive determination of *B. pseudomallei* in the early period of infection is urgently required.

In recent years, ECL biosensor has been verified to be an alternative platform for bacteria sensing, resulting from the merits of simple operation and low cost [11–13]. N-(4-aminobutyl)-N-(ethylisoluminol) (ABEI), as a typical derivative of luminol, has been widely used in the ECL reaction system (ABEI/ H_2O_2) due to its good stability, low toxicity, and easy modification [14–16]. Given the consensus that nanozymes can significantly enhance the ECL emission of ABEI/ H_2O_2 by loading enormous ABEI and catalyzing H_2O_2 to produce ROSs owing to their high surface area and good peroxidase-like activity, various nanozymes have been employed [17–20]. For instance, Yuan' group proposed ABEI functionalized Fe-MIL-101 that is one of metal-organic frameworks (MOFs), revealing an enhanced ECL performance [21]. Furthermore, Jin' group fabricated luminol-capped Ag nanoparticles (NPs)@Co-MOF, demonstrating that metal-doped MOF could further improve the ECL intensity of luminophore [22].

Inspired by these valuable reports, we speculate that Au NPs coated Co-MOF is capable of enhancing the emission of ABEI, which contributes to develop high-performance ECL biosensors.

Biological systems take advantage of highly complicated and hierarchical machineries to operate various functions [23, 24]. Inspired by natural phenomenon, 3D DNA walking nanomachines have been designed and applied in the field of biosensing for the rapid transduction and amplification of signals because of the increased local concentration of DNA strands [25–27]. Usually, the autonomous motion of the nanomachines is propelled by DNAzyme, protease, and strand displacement [28–30]. It is worth noting that the signal amplification ability of DNAzyme and strand displacement-propelled nanomachines is restricted owing to the low catalytic efficiency of DNAzyme and the random collision of drive strand and track [31, 32]. In contrast, protease-powered nanomachines are promising alternatives for constructing 3D magnetic walking nanomachines that can achieve rapid walking processivity and avoid the interference from environmental factors.

Herein, combining 3D Exo III-propelled magnetic walking nanomachine and Au@Co-MOF@ABEI nanocubes, a pragmatic ECL biosensing platform for an ultrasensitive detection of *B. pseudomallei* was established (Scheme 1). For process A, multifunctionalized Au@Co-MOF nanocubes were synthesized by a simple wet-chemical approach and then used to loading numerous ABEI for forming Au@Co-MOF@ABEI nanocubes. For process B, the designed 3D magnetic walking nanomachine consisted of three aspects: the biotin-modified DNA duplex probes with the exposed 3' end (PP/DWT as a walker and S3/DWT1 as DNA track) immobilized on the same magnetic bead (MB), target *B. pseudomallei* gene as energy input, and Exo III as a driver. The presence of target gene triggered the release of the DWT from PP/DWT duplex, which could then displace S3 to form DWT/DWT1 with blunt 3'end. Subsequently, Exo III digested the DWT1 of DWT/DWT1 and released the DWT again. In this way, the DWT autonomously moved along the designed 3D track, resulting in the release of numerous S3. For process C, the ECL biosensor was fixed on the glassy carbon electrode (GCE) which was deposited with Au@Co-MOF@ABEI nanocubes to achieve a high ECL emission ("signal-on" state). Subsequently, dopamine-labeled S2 strands (S2-DA) bound with S1 to sufficiently quench the initial ECL signal ("signal-off" state). Finally, the released S3 displaced S2-DA to recover the quenched ECL signal ("signal-on" state). Benefiting from Au@Co-MOF@ABEI nanocubes and 3D magnetic walking nanomachine, the ECL biosensor for *B. pseudomallei* gene determination exhibited remarkable analytical performance, which provided a novel platform for pathogenic bacteria determination.

Experimental Section

Materials and reagents

Streptavidin-labeled MB (SMB, 1 μm in diameter, 10 mg mL^{-1}) was obtained from Selvinth Biotechnology Co., Ltd. (Chengdu, China). Exo III was purchased from New England Biolabs (Beijing, China). 3-amino-propyl triethoxysilane (APTES), Polyvinyl pyrrolidone (PVP), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and DA were obtained from Sigma-

Aldrich Co., Ltd. (St. Louis, MO, USA). Cobalt nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), chloroauric acid hexahydrate ($\text{HAuCl}_4 \cdot 6\text{H}_2\text{O}$), and tripotassium hexacyanocobaltate $\text{K}_3[\text{Co}(\text{CN})_6]$ were purchased from Aladdin (Shanghai, China). ABEI and MES hydrate ($\text{C}_6\text{H}_{13}\text{NO}_4\text{S}$) were obtained from Macklin Inc. (Shanghai, China). *B. pseudomallei* (HNBP001) was obtained from the Hainan Medical University. *Staphylococcus aureus* (*S. aureus*, ATCC26213) and *Escherichia coli* (*E. coli*, ATCC25922) were provided by the Ninth People's Hospital of Chongqing. Other reagents were supplied by Chemical Reagent Co., Ltd. (Chongqing, China).

The oligonucleotide DNAs were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). All sequences are listed in Table S1 and S2. The buffers employed in the present work were showed as follows: TE buffer (10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0). Tris-HCl buffer (pH 7.4) contained 20 mM Tris, 14 mM NaCl, 5 mM KCl, 1 mM MgCl_2 , and 1 mM CaCl_2 . Washer buffer 1 (pH 7.4) contained 50 mM Tris, 1 M NaCl, and 0.5 mM EDTA. Washer buffer 2 (pH 7.4) contained 20 mM Tris, 140 mM NaCl, 5 mM KCl, 1 mM MgCl_2 , 1 mM CaCl_2 , and 0.05% Tween-20. All solutions were stored at 4 °C. All other chemicals were of analytical grade, and ultrapure water from a Millipore water purification system ($\geq 18.2 \text{ M}\Omega$, MA, USA) was used in the all experiments.

Apparatus

The multifunctional MPI-E analyzer (Xi'An Remax Electronic Science & Technology Co., Ltd. Xi'An, China) was utilized to carry out ECL analysis with the voltage of the photomultiplier tube at 800 V. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) measurements were taken with a CHI660D electrochemistry workstation (Shanghai Chenhua Instruments, China). Three kinds of electrodes were included in the measuring system, referred to a modified GCE (working electrode), a platinum wire (counter electrode), and an Ag/AgCl electrode (reference electrode). Fluorescence signals were obtained using the Cary Eclipse Fluorescence spectrophotometer (Agilent, California). The PCR analysis was performed with a Cycler thermal cycler (Bio-Rad Laboratories, USA). X-ray photoelectron spectroscopy (XPS) spectra was registered with an Thermo ESCALAB 250Xi (USA). X-ray diffraction (XRD) patterns were gained with a D8 ADVANCE X-ray diffractometer (Bruker, Germany) to confirm the structure of the materials. The morphology characterization of different nanomaterials was characterized by Oxford x-max energy dispersive spectroscopy (EDS, Berlin, Germany) and SU8010 scanning electron microscopy (SEM, Berlin, Germany).

Fabrication of 3D magnetic walking nanomachine

First, 10 μL of biotin-modified DNA walker stand (DWT, 10 μM) and 10 μL of protecting probe (PP, 10 μM) were dissolved in the 100 μL of 20 mM Tris-HCl buffer at 37 °C for 1 h to form PP/DWT duplex strand (PWT, 1 μM). Meanwhile, 10 μL of biotin-modified DNA track stand (DWT1, 10 μM) and 10 μL of output stand (S3, 10 μM) were also dissolved in the 100 μL of 20 mM Tris-HCl buffer at 37 °C for 1 h to form S3/DWT1 double-stranded track probes (SDs). Second, 10 μL of SMB was washed three times with washer buffer 1 and then redispersed in 10 μL of 20 mM Tris-HCl buffer. Third, to combine DNA strands with SMB, 100 μL of PWT and SDs at the molar ratio of 1 to 20 was sufficiently mixed with 10 μL of the

redispersed SMB for 1 h at 37°C. Subsequently, the formed PWT-SDs-SMB was successively washed three times with 20 mM Tris-HCl buffer after magnetic separation. Then, the 3D magnetic walking nanomachine was firstly initiated by adding 10 μL of target gene with various concentrations, 5 μL of PWT-SDs-SMB, 10 μL of 10 $\times$ NEBuffer 1 (100 mM bis Tris Propane-HCl, 100 mM MgCl_2 , 10 mM dithiothreitol), and 72.5 μL of ultrapure water for 30 min at 37°C. After that, 2.5 μL of 100 U mL^{-1} Exo III was mixed for brief vortex and further incubated at 37°C for 1.5 h to perform the motion of walker. Finally, numerous S3 were obtained after magnetic separation, and the Exo III was inactivated at 70 °C for 30 min.

Preparation of Au@Co-MOF@ABEI nanocubes

The preparation steps of Co-MOF were described according to previously reported method with minor alteration [33]. Typically, 5 mL of aqueous solution A was obtained by mixing up 0.0375 g $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 0.0551 g $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$, and 5 mL of ultrapure water. Meanwhile, 7.5 mL of aqueous solution B was acquired by adding 0.033 g $\text{K}_3[\text{Co}(\text{CN})_6]$ into 7.5 mL of ultrapure water. Then, solution A and solution B were mixed for brief ultrasound at room temperature (RT) overnight. In the following, the precipitate with pink color was washed with absolute ethanol by centrifugation (12000 rpm, 5min) at least three times. Finally, the pink Co-MOF powders were collected after drying at 60 °C for 2 h.

The preparation of Au@Co-MOF@ABEI nanocubes was depicted as follows. In brief, the synthesized Co-MOF (0.025 g) and 1 ml of APTEs were mixed into 20 mL of ultrapure water and stirred for 1.5 h to decorate Co-MOF with abundant amino groups. After that, 0.25 mL of $\text{HAuCl}_4 \cdot 6\text{H}_2\text{O}$ (1.0 wt%) and 0.00125 g PVP were mixed into the preceding solution and stirred for 15 min. In the following, 10 μL of fresh NaBH_4 (1.9 M) was dropped into the mixed solution and stirred for 1.5h to obtain Au@Co-MOF. Subsequently, the resulted Au@Co-MOF was obtained by centrifugation and washed with absolute ethanol for three times. After that, the Au@Co-MOF was re-suspended in 20 mL of ultrapure water. Then, 1 mL of ABEI (10 mM) was added into the Au@Co-MOF solution with full stirring for 30 min. The mixed solution was washed with ultrapure water to eliminate unreacted ABEI. Finally, the obtained Au@Co-MOF@ABEI nanocubes were dissolved in 0.1% chitosan solution and kept at 4°C for further use.

Preparation of S2-DA

The S2-DA was synthesized as follows. Firstly, 0.0192 g EDC and 0.0115 g NHS were dissolved in 1.0 mL of MES buffer (0.1 M, pH 6.0), and their final concentration was 100 mM. Second, 5 μL of the carboxyl-modified DNA (S2, 100 μM) was mixed into 145 μL of PBS (0.1 M, pH 7.4). Then, the S2 strand was activated carboxyl using 40 μL of EDC (100 mM) and 10 μL of NHS (100 mM) for 2 h at 4°C. Finally, the S2-DA composite was obtained by mixing 200 μL of 100 μM DA solution with the preceding solution and stirring overnight at 4°C.

Fabrication of the switching ECL biosensor

Prior to use, GCE was polished with 0.3 and 0.05 μm Al_2O_3 slurry orderly, and sonicated with ultrapure water to attain a mirror-like surface. After drying in the nitrogen, the cleaned GCE was decorated by 10 μL

of Au@Co-MOF@ABEI nanocubes and dried at RT to form nanofilm. Then, 10 μL of S1 (1 μM) was added to the modified electrode at 4°C overnight. Afterward, the modified electrode was incubated with 5 μL of MCA (1 μM) to block the remaining active sites for 30 min. Subsequently, 10 μL of S2-DA was dropped onto the modified electrodes at 37°C for 1 h. After the hybridization between S1 and S2-DA, the excess S2-DA was eliminated by the washer buffer 2. Then, 10 μL of the released S3 was hybridized with the S2-DA for 1.5 h. Washer buffer 2 was used to clean the electrode after each construction step, respectively, and the obtained electrode was preserved at 4°C for later use. Finally, the established ECL biosensor was tested in 2 mL PBS (0.1 M, pH 8.0) including H_2O_2 (3 mM) to record the ECL signal.

Results And Discussion

Characterization of Au@Co-MOF@ABEI nanocubes

The size and surface morphologies of the as-synthesized nanomaterials were characterized by SEM. As showed in Fig. 1A, the Co-MOFs illustrated a nanocube with a side length of about 500 ± 10 nm and a smooth surface, which was favorable for further modification. Fig. 1B showed that Au NPs were coated on Co-MOFs and had an average diameter of 14 ± 2 nm (Fig. 1C), which agreed with the typical XRD pattern of Co-MOF and Au@Co-MOF (Fig. S1). These results indicated the successful synthesis of Au@Co-MOF@ABEI nanocubes. In addition, the element distribution of Au@Co-MOF@ABEI nanocubes was verified by EDX elemental mapping with the high-angle annular dark-field scanning electron microscopy (HAADF-SEM) in Fig. 1D. As shown in Fig. 1(E-I), the occurrence of C, N, O, Au, and Co elements showed that Au NPs on the surface of Co-MOFs were successfully synthesized. Subsequently, XPS analysis was utilized to further investigate the electronic state and elemental composition of the Au@Co-MOF@ABEI. As illustrated in Fig. 1(J-L), the peaks at 285.0, 398.4, and 531.8 eV belonged to C1s, N1s, and O1s, respectively. The characteristic peaks at 84.3 eV and 88.0 eV were originated from Au 4f peaks, manifesting that the metallic Au adhered to the surface of the nanocubes [34]. As revealed in the high-resolution spectra of Co 2p, the characteristic peaks at 799.9, 797.1, 784.4, and 781.9 eV could be indexed to metallic Co [35], which was in accord with the SEM-EDX mapping results, further indicating that Au@Co-MOF@ABEI nanocubes were successfully synthesized as expected.

The mechanism of the ECL biosensor

To confirm the superiority of the Au@Co-MOF@ABEI nanocubes in ECL intensity, a contrast experiment was implemented by comparing the performance of Au@Co-MOF@ABEI to those of Co-MOF@ABEI, Au@ABEI, and ABEI. As illustrated in Fig. 2A, the ECL intensity obtained by diverse MOF-based nanomaterials followed the order of Au@Co-MOF@ABEI nanocubes > Co-MOF@ABEI > Au@ABEI > ABEI. Specifically, only a weak ECL signal (1648 a.u.) was detected when ABEI was dropped onto the cleaned electrode (curve a), the Au@ABEI-modified GCE produced ECL signal about 3293 a.u. (curve b), and the Co-MOF@ABEI-modified GCE generated ECL signal about 6872 a.u. (curve c). However, when Au@Co-MOF@ABEI nanocubes was preferred to act as ECL luminophore, a remarkably higher ECL signal (12392

a.u.) was observed by the ECL biosensor (curve d). These results indicated that the multifunctionalized Au@Co-MOF@ABEI nanocubes containing Au NPs and Co-MOF could greatly enhance the ECL of ABEI.

In addition, the “on-off-on” mode was investigated. As displayed in Fig. 2B, the Au@Co-MOF@ABEI nanocubes was firstly immobilized on the electrode surface to obtain an enhanced ECL signal (12362 a.u.), owing to the decomposition of Au@Co-MOF toward H_2O_2 to produce many ROSs including superoxide radical ($\text{O}_2^{\cdot-}$) and hydroxyl radical ($\text{OH}\cdot$) that could further react with ABEI (“signal-on” state). Next, S1/S2-DA duplex probes were dropped onto the modified electrode, leading to the obvious decrease of the initial ECL signal (1692 a.u.), resulting from the quenching effect of DA toward ABEI luminescence (“signal-off” state). Then, S2-DA was replaced by the output S3, leading to the recovery of the ECL response (9540 a.u.), indicating that the 3D magnetic walking nanomachine was successfully fabricated (“signal-on” state). Therefore, these results indicated that the established “on-off-on” ECL biosensor possessed the advantages of excellent ECL performance and obvious signal-to-noise ratio (S/N).

Electrochemical characterization of the ECL biosensor

CV and EIS were used to investigate the stepwise modification process of the ECL biosensor in $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (5.0 mM) including KCl (0.1 M). As displayed in Fig. 2C, an initial CV peak was obtained on the bare GCE (curve a). When the Au@Co-MOF@ABEI (curve b) was immobilized onto the electrode, the CV peak decreased, given a poor conductivity of the nanocubes as an ECL luminophore. After the S1 was immobilized onto the GCE/Au@Co-MOF@ABEI, the current signal decreased due to the electronic repulsion between the negatively charged DNA and $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (curve c). As expected, the current response was greatly reduced after hybridizing with the S2-DA (curve d), because there was repulsive effect from abundant S2-DA. Subsequently, peak current dramatically increased and characteristic peak gap was significantly separated (curve e), because S3 was dropped on the prepared electrode to displace S2-DA, which largely decreased negative charges of the electrode surface. The CV results were fairly consistent with the results obtained from EIS (Fig. 2D), in which the R_{et} values changed upon the immobilization, hybridization, and displacement process. These results indicated the feasibility of the constructed biosensor.

Optimization of the experimental conditions

Firstly, the 3D magnetic walking nanomachine was successfully fabricated and verified the feasibility by fluorescent experiment (Fig. S2). Then, experimental parameters of the walking nanomachine were optimized (Fig. S3A and 3B). On this basis, to obtain excellent analytical capability for *B. pseudomallei* gene detection, different experimental conditions of the ECL biosensor were further optimized. the ECL intensity and S/N were used to evaluate the performance of the developed biosensor. As shown in Fig. 3A, the ECL intensity and S/N for 10.0 nM target gene increased with the increasing the molar ratio of PWT to SDs and then decreased obviously after 1:20, which was then chosen as the optimal ratio. As could be seen in Fig. 3B, the ECL intensity and S/N increased with the increasing concentration of Exo III in the range from 100 to 250 U mL^{-1} , and then showed a stable tendency. Therefore, 250 U mL^{-1} was used as the optimized concentration of Exo III.

Furthermore, the concentration of DA was vital in the quenching effect of the ECL responses. As exhibited in Fig. 3C, after introducing 0.31 μM DA, the S/N was maximum. As shown in Fig. 3D, the incubation time of S3 and S2-DA was studied, and the results indicated that the ECL signals increased with the incubation time from 0.5 to 1.5 h, while the signal slightly increased when the incubation time was longer than 1.5 h. Therefore, 1.5 h was selected as the optimized time.

Analytical performance of the ECL biosensor

Under the optimal experimental conditions, the dependence of ECL intensity was investigated to evaluate the analytical performance of the ECL Biosensor. Fig. 4A showed the good linear relationship between the ECL intensity and concentration of *B. pseudomallei* gene in the range of 0, 100.0 aM to 100.0 pM (curve a-h). As shown in Fig. 4B, The corresponding calibration plot between the ECL intensity and the logarithm (lg) of the *B. pseudomallei* gene concentration showed a linear regression equation of $I = 1362.9 \lg c - 627.9$ ($R^2 = 0.9958$). The limit of detection (LOD) of 60.3 aM was evaluated based on a $3\sigma_b/\text{slope}$, the σ_b stands for the standard deviation of three blank samples, which was about 2 orders of magnitude lower than that (4.97 fM) of the individual ECL system without the nanomachine (Fig. S4A and B). In addition, the LOD of the integral ECL platform was about 5 orders of magnitude lower than that (0.34 pM) of the individual walking nanomachine (Fig. S4C and D). The ECL biosensor exhibited a lower LOD, resulting from multifunctionalized Au@Co-MOF@ABEI nanocubes and effective magnetic walking nanomachine. Moreover, these results indicated that the “off-on” mode possessed the superiorities of high sensitivity, obvious S/N, and wide range of detection. Therefore, the constructed ECL biosensor was a versatile tool for an ultrasensitive detection of *B. pseudomallei* gene.

The stability of the ECL biosensor is important for clinical diagnosis. As showed in Fig. 4C, an ECL signal for target gene within 100 s was obtained with the relative standard deviation (RSD) of 2.15%, manifesting that the biosensor obtained eminent stability. In order to evaluate the specificity of the constructed ECL biosensor, nine diverse mismatched synthetic DNAs were examined. As shown in Fig. 4D, the ECL intensity for T1 was the highest among other mismatched DNAs, containing T1 (1-base mismatch), T2 (2-base mismatch), T3 (3-base mismatch), T4 (4-base mismatch), T5 (5-base mismatch), T6 (6-base mismatch), T7 (7-base mismatch), T8 (8-base mismatch), and T9 (9-base mismatch). The ECL intensity for T9 was close to that for the blank space. These results indicated that the constructed ECL biosensor could discriminate different mismatched DNA effectively and showed good selectivity.

Real samples analysis

To evaluate whether the constructed ECL biosensor could detect practical sample, sensitively and specifically, the *B. pseudomallei* were cultivated into human serum at the concentrations from 0 to $1.5 \times 10^8 \text{ CFU mL}^{-1}$. After a preprocessing step for the human serum samples, the PCR analysis was then executed by using extracted genomic DNA for each concentration of *B. pseudomallei*. Meanwhile, the established ECL biosensor was also carried out to detect the denatured PCR products of *B. pseudomallei*. The ECL intensity of the constructed ECL biosensor to different PCR samples were illustrated in Fig. 5A. As seen in Fig. 5B, the linear regression equation between the ECL responses and *B. pseudomallei*

concentration was expressed as $I = 912.82 c + 766.20$, in which I represents for ECL intensity, c stands *B. pseudomallei* concentration (CFU mL⁻¹) with a correlation coefficient of 0.9994. The ECL biosensor could analyze *B. pseudomallei* down to 9.0 CFU mL⁻¹ in real serum samples, which was much lower than that of previous methods for pathogenic bacteria analysis (Table S3). Therefore, The established biosensing platform showed high sensitivity, specificity, and speediness, exhibiting the potential as a pragmatic tool for *B. pseudomallei* determination in real samples.

The stability of the ECL biosensor for *B. pseudomallei* detection was an important factor for clinical diagnosis in real samples analysis. As illustrated in Fig. 5C, the stability of the ECL biosensor was studied by using the RSD of the ECL intensity for five concentrations (1.5 x 10¹ CFU mL⁻¹, 1.5 x 10² CFU mL⁻¹, 1.5 x 10³ CFU mL⁻¹, 1.5 x 10⁴ CFU mL⁻¹, and 1.5 x 10⁵ CFU mL⁻¹) of *B. pseudomallei* (a-e). The RSD were calculated to be 4.02%, 0.84%, 0.84%, 1.55%, and 0.21% (n = 3). This result demonstrated that the developed ECL biosensor possessed good stability. In addition, to evaluate the specificity of constructed ECL biosensor, the PCR products for different types of pathogenic bacteria were detected, including 1.5 x 10⁸ CFU mL⁻¹ of blank, *E. coli*, *S. aureus*, mixture and *B. pseudomallei*, respectively. As shown in Fig. 5D, the ECL intensity of *B. pseudomallei* detection was much larger than those of other detection, while the intensity of *E. coli* and *S. aureus* detection were close to that of blank, and these results indicated that the constructed ECL biosensor showed good specificity for an ultrasensitive detection of *B. pseudomallei*. Therefore, the high stability and specificity could further confirm the practicality of the developed biosensor.

Conclusions

In this work, an “off-on” ultrasensitive ECL biosensor have been successfully constructed for *B. pseudomallei* detection combined with the multifunctionalized Au@Co-MOF@ABEI nanocubes and 3D magnetic walking nanomachine. The nanocubes could not only immobilize enormous ABEI but exhibit high peroxidase-like activity toward H₂O₂, resulting in the enhanced ECL signals. Meanwhile, the robust walking nanomachine efficiently driven by Exo III endowed the ECL biosensor with high S/N ratio. Moreover, the developed ECL biosensor could detect *B. pseudomallei* in serum samples. As expected, the constructed ECL biosensor for *B. pseudomallei* detection exhibited high sensitivity, good specificity, and well stability, possessing the great potential applications in the early diagnosis and treatment of melioidosis.

Abbreviations

B. pseudomallei: *Burkholderia pseudomallei*; ECL: Electrochemiluminescence; ROSs: Reactive oxygen species; Exo III: Exonuclease III; PCR: Polymerase chain reaction; ABEI: N-(4-aminobutyl)-N-(ethylisoluminol); MOFs: Metal-organic frameworks; NPs: Nanoparticles; MB: Magnetic bead; GCE: Glassy carbon electrode; DA: dopamine; APTES: 3-amino-propyl triethoxysilane; PVP: Polyvinyl pyrrolidone; EDC: N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride; NHS: N-hydroxysuccinimide; *S. aureus*:

Staphylococcus aureus; *E. coli*: *Escherichia coli*; EDTA: Ethylenediaminetetraacetic acid; CV: Cyclic voltammetry; EIS: Electrochemical impedance spectroscopy; XPS: X-ray photoelectron spectroscopy; XRD: X-ray diffraction; EDS: Energy dispersive spectroscopy; SEM: Scanning electron microscopy; DWT: DNA walker stand; PP: Protecting probe; HAADF: High-angle annular dark-field; S/N: Signal-to-noise ratio; RSD: Relative standard deviation; LOD: Limit of detection.

Declarations

Authors' contributions

Y. Wang, B. Shen, C. Li, H. Wu, and Y. Wang conducted all ECL experimental work, data analysis, and writing manuscript. S. Tian, X. Li, N. Luo, and R. Liu collected clinical samples. X. Li, J. Chen, and W. Cheng performed manuscript revisions. S. Ding executed project guidance. C. Zhu and Q. Xia provided funding and data curation. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated and analyzed during this study are included in this article and additional file. The additional file is available. The DNA sequences employed in the present work (Table S1). Sequences of primer and target in PCR (Table S2). Preparation of pure microbial sample and artificial specimen (Experimental section). XRD Pattern of Co-MOF and Au@Co-MOF (Fig. S1). Schematics of the 3D magnetic walking nanomachine and fluorescence intensity of 3D magnetic walking nanomachine incubated with target DNA and without target DNA (Fig. S2). Optimization of the 3D magnetic walking experimental parameters: the molar ratio of PWT and SDs covered on the SMB and the concentration of Exo III (Fig. S3). ECL intensity at different target gene concentrations, the calibration curve between the logarithm of target gene concentrations and ECL intensity, the 3D magnetic walking nanomachine for the detection of target DNA, and the calibration curve between the logarithm of target gene concentrations and FL intensity (Fig. S4). Comparison with different biosensors for bacteria detection (Table S3).

Ethics approval and consent to participate

This study has been approved by the ethics committee of Hainan Medical University and conducted in accordance with ethical guidelines.

Consent for publication

All authors have provided consent for the manuscript to be published.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

SEM images of (A) Co-MOF and (B and C) Au@Co-MOF@ABEI nanocubes (inset: the size distribution of Au NPs). (D) HAADF-SEM image of Au@Co-MOF@ABEI. SEM-EDS elemental mappings of (E) C, (F) N, (G) O, (H) Au, and (I) Co. XPS spectra for (J) the full region of XPS of Au@Co-MOF@ABEI nanocubes. XPS analysis of (K) Co 2p and (L) Au 4f.

Figure 2

(A) ECL intensity of (a) ABEI, (b) Au@ABEI, (c) Co-MOF@ABEI, and (d) Au@Co-MOF@ABEI nanocubes. (B) Schematic diagram of the DA-driven light switching biosensor in 2 mL PBS (0.1 M, pH 8.0) including 3 mM H_2O_2 . (C) EIS plots and (D) CV curves of (a) bare GCE, (b) GCE/Au@Co-MOF@ABEI, (c) GCE/Au@Co-MOF@ABEI/S1, (d) GCE/Au@Co-MOF@ABEI/S1/MCA/S2-DA, and (e) after adding into released S3, measured in 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ containing 0.1 M KCl. The inset was the equivalent circuit for EIS.

Figure 3

The optimization of (A) the molar ratio of PWT and SDs, (B) the concentration of Exo III, (C) DA, and (D) the incubation time of S3 and S2-DA, when one parameter changes while the others are under their optimal conditions. Error bars: SD, $n = 3$.

Figure 4

(A) The ECL intensity of the constructed biosensor hybridized with different concentrations of target gene (0, 100.0 aM, 1.0 fM, 10.0 fM, 100.0 fM, 1.0 pM, 10.0 pM, and 100.0 pM). (B) The calibration curve of the biosensor. (C) The stability of the ECL biosensor for detecting 10 nM target DNA. (D) ECL intensity for T1, T2, T3, T4, T5, T6, T7, T8, T9, and blank.

Figure 5

(A) The ECL intensity of the constructed ECL biosensor hybridized with different concentrations of *B. pseudomallei* (0 , 1.5×10^1 CFU mL⁻¹, 1.5×10^2 CFU mL⁻¹, 1.5×10^3 CFU mL⁻¹, 1.5×10^4 CFU mL⁻¹, 1.5×10^5 CFU mL⁻¹, 1.5×10^6 CFU mL⁻¹, 1.5×10^7 CFU mL⁻¹, and 1.5×10^8 CFU mL⁻¹). (B) Correlation curves of ECL intensity as a function of *B. pseudomallei*. (C) Investigation of the stability of the ECL biosensor: ECL intensity responding to different concentrations of *B. pseudomallei* (1.5×10^1 CFU mL⁻¹, 1.5×10^2 CFU mL⁻¹, 1.5×10^3 CFU mL⁻¹, 1.5×10^4 CFU mL⁻¹, and 1.5×10^5 CFU mL⁻¹) of *B. pseudomallei* (a-e). (D) the selectivity of biosensor against different interfering substances: *E. coli* (1.5×10^8 CFU mL⁻¹), *S. aureus* (1.5×10^8 CFU mL⁻¹), mixture with 1.5×10^8 CFU mL⁻¹ interfering substances, and *B. pseudomallei* (1.5×10^8 CFU mL⁻¹).

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